Human Genes and Influenza

Samira Mubareka[1] and Peter Palese[1,2]

[1]Department of Microbiology and [2]Division of Infectious Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, New York

(See the article by Albright et al., on pages 18–24.)

Why some individuals resist infection or recover quickly, whereas others experience severe disease associated with infection, is a fundamental question that medicine has struggled to answer. Pathogens and host immune factors have been extensively investigated for many infectious diseases, to address these questions. However, limited information is available concerning the influence of host genetics on the response to viral infections. Genetic determinants have the potential to play a role at numerous points during the course of viral infection, including viral attachment and entry, replication, disease progression and development of severity, and, finally, transmission. In this issue of the *Journal,* Albright et al. [1] propose that the severity of influenza illness may have a heritable component. To investigate this hypothesis, the authors used as a resource the Utah Population Data Base, which contains data from founding families and their descendants, comprised primarily of members of the Church of Jesus Christ of Latter-Day Saints (i.e., Mormons), thus representing a relatively ethnically homogeneous population.

Albright et al. [1] estimated the relative risk (RR) of death for relatives of 4855 individuals (spanning ≥3 generations) who died of influenza. A substantial proportion of deaths occurred during the 1918 influenza pandemic, when a total of 1937 deaths were documented between 1918 and 1921, and 1293 additional deaths occurred between 1922–1932, followed by a dramatic decrease in the number of deaths occurring annually.

The RR of death for first-degree relatives was 1.54 ($P < .0001$). The RR was higher for spouses (1.98) and for second- and third-degree relatives (1.22 and 1.16, respectively). The timing of the deaths of third-degree relatives suggests that the deaths were not the result of a common exposure. To control for shared environment, the RR of death for spouses' relatives was compared and was found to be lower for first-, second-, and third-degree relatives.

Excess relatedness among individuals dying of influenza was estimated using the Geneological Index of Familiality, which demonstrated that relatedness among these individuals, including individuals who died during the 1918 pandemic, was greater than expected ($P < .001$). The analysis was repeated with close relatives excluded (to control for shared environment), and the results were consistent with previous findings. Consistent results were not observed when the same analysis was repeated for individuals with diphtheria-associated deaths. For such individuals, excess relatedness was demonstrated; however, when close relationships were excluded, no excess relatedness was detected.

Specific genes responsible for the host immune response have been invoked as major determinants of the clinical course of HIV-associated disease and hepatitis B and C virus infections [2, 3]. However, there is very little information with respect to genetic determinants as they relate to severe influenza. Over the past decade, a greater understanding of the immune evasion strategies of influenza virus has developed. This knowledge can be used to propose several candidate genes that may be responsible for severe illness. Clinical and animal studies indicate that cytokine dysregulation is associated with acute respiratory distress syndrome and death among hosts infected with avian influenza virus (H5N1) [4–6].

Toll-like receptors (TLRs), particularly TLR3 (which recognizes double-stranded RNA) and TLR7 and TLR8 (which recognize single-stranded RNA), are central to antiviral innate immunity [7]. Single-nucleotide polymorphisms in TLR genes are not uncommon and vary among populations [8]. TLR4 has been implicated in the innate immune response to respiratory syncytial virus (RSV) infection, and polymorphisms in the *TLR4* gene have

been associated with severe bronchiolitis in RSV-infected infants, although the significance of the Asp299Gly polymorphism appears to be a matter of ongoing debate [9–12]. A missense mutation in the *TLR3* gene (F303S) conferring loss of function in in vitro assays has been identified in a Japanese child with influenza-associated encephalopathy [13]. Interestingly, better survival rates have been demonstrated for *TLR3* knockout mice than for wild-type mice, despite the *TLR3* knockout mice having higher lung virus titers after influenza virus infection [14]. *TLR* genes (in particular, *TLR3*) would therefore be worthy candidate genes to investigate. In addition, RNA helicases, such as retinoic acid inducible gene I (RIG-I), recognize double-stranded RNA and, thus, contribute to the antiviral state of an infected cell [7]. Inhibition of RIG-1 by nonstructural protein 1 (NS1) of influenza virus antagonizes interferon (IFN) production and is a means by which the virus evades the host immune system [15]. Therefore, the *RIG-I* gene should also be considered to be a candidate for attempts to elucidate aberrations in the immune system that may lead to severe influenza illness.

Other host gene products involved in IFN production and induction antagonized by the influenza virus NS1 are worthy of consideration [16]. Changes in genes involved in other aspects of immunity, such as the complement system, have also been associated with recurrent viral upper respiratory tract infections, and they should not be overlooked [17]. Mannose-binding lectins are part of the innate immune system, binding pathogen-associated carbohydrates and activating the complement system through the classical pathway. Mutations within the *MBL* gene have been associated with severe acute respiratory syndrome (SARS) [18]. Susceptibility to the SARS coronavirus has been associated with changes in the 2'5'-oligoadenylate synthetase 1 *(OAS1)* and myxovirus resistance 1 *(MxA)* genes as well [19]. All of these genes would be excellent candidates for an analysis to identify determinants of severity of disease after influenza virus infection.

The findings of the study by Albright et al. [1] raise many complex questions. The outcome of influenza illness is likely to be dependent on environmental, nutritional, demographic, immunological, and (especially) virologic factors. Undoubtedly, host factors, including comorbidities, will also have a bearing on outcome, and the relative contribution of different genetic determinants is likely to vary depending on environmental and virologic contexts. A significant proportion of the data collected in this study emanates from the influenza pandemic of 1918, which occurred in a setting very different from that of interpandemic cases. In addition, the use of death certificates imposes significant limitations. During the 1918 pandemic, anyone dying with respiratory symptoms was likely to have been given a code denoting "pneumonia and influenza," which introduced a potential bias. One could argue that errors related to certification of death would be random rather than systematic and should have minimal bearing on the results. Nonetheless, death certificates are notoriously inaccurate, and the absence of microbiological and/or serologic data is a significant limitation of this study.

There are also a number of potential confounding factors in this investigation. Other heritable factors, such as cardiopulmonary conditions or cystic fibrosis (the most common autosomal recessive lethal disorder in whites), are possible examples. Other examples would include common conditions that have a genetic component, such as asthma and coronary artery disease. There is reasonable evidence that the influenza-associated mortality rate is higher for individuals with established coronary artery disease (than for those with other chronic conditions) [20]. Coinfections may also confound the data, and the contribution of coinfecting pathogens to mortality cannot be excluded, bearing in mind that genetic susceptibility to such organisms as the pneumococcus has been proposed [21].

Environmental and geographic factors, such as socioeconomic variables and environmental and occupational (e.g., mining) exposures, may also cluster in families, and such factors as poverty, malnutrition, and overcrowding may span generations. In addition, access to health care may have been limited for certain families in remote settings, thus contributing to the lethality of influenza virus infection, particularly in individuals at the extremes of age.

The implications of this study by Albright et al. [1] are that heritable factors for severe influenza illness may well exist and that elucidation of these factors remains a challenge. Identification and characterization of genetic determinants of the outcome of infection with influenza virus would lead to further insight into the pathophysiology of the virus and its immune evasion strategies. One would hope that this insight would translate into identification of prognostic factors and improved management of individuals at risk for adverse outcomes after influenza virus infection. Identification of genetic determinants of outcome after influenza virus infection would require a substantial number of severe cases of influenza (in which severity of illness is not the result of other factors, such as underlying cardiovascular disease) to gain sufficient power and produce interpretable data. Resources stemming from the sequencing of the human genome are now available and render this challenging task more feasible. Such approaches as the genome-wide and candidate gene study designs are reviewed by Burgner et al. elsewhere [22]. The latter approach may be more feasible, given the growing knowledge regarding IFN antagonism and immune evasion by influenza viruses. One must bear in mind that findings may not apply across all host or virus populations. In the event of a pandemic, however, early identification of and intervention for individuals at higher risk for severe illness and death resulting from influenza due to a predisposing genetic determinant would be invaluable.

References

1. Albright FS, Orlando P, Pavia AT, Jackson GG, Cannon Albright LA. Evidence for heritable predisposition to death due to influenza. J Infect Dis **2007**; 197:18–24 (in this issue).
2. O'Brien SJ, Nelson GW. Human genes that limit AIDS. Nat Genet **2004**; 36:565–74.
3. Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. World J Gastroenterol **2007**; 13:1770–87.
4. Xu T, Qiao J, Zhao L, et al. Acute respiratory distress syndrome induced by avian influenza A (H5N1) virus in mice. Am J Respir Crit Care Med **2006**; 174:1011–7.
5. Szretter KJ, Gangappa S, Lu X, et al. Role of host cytokine responses in the pathogenesis of avian H5N1 influenza viruses in mice. J Virol **2007**; 81:2736–44.
6. de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med **2006**; 12:1203–7.
7. Meylan E, Tschopp J. Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. Mol Cell **2006**; 22:561–9.
8. Cheng P-L, Eng H-L, Chou M-H, You H-L, Lin T-M. Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population. Transl Res **2007**; 150:311–8.
9. Haynes LM, Moore DD, Kurt-Jones EA, Finberg RW, Anderson LJ, Tripp RA. Involvement of toll-like receptor 4 in innate immunity to respiratory syncytial virus. J Virol **2001**; 75:10730–7.
10. Puthothu B, Forster J, Heinzmann A, Krueger M. TLR-4 and CD14 polymorphisms in respiratory syncytial virus associated disease. Dis Markers **2006**; 22:303–8.
11. Tal G, Mandelberg A, Dalal I, et al. Association between common Toll-like receptor 4 mutations and severe respiratory syncytial virus disease. J Infect Dis **2004**; 189:2057–63.
12. Paulus SC, Hirschfeld AF, Victor RE, Brunstein J, Thomas E, Turvey SE. Common human Toll-like receptor 4 polymorphisms—role in susceptibility to respiratory syncytial virus infection and functional immunological relevance. Clin Immunol **2007**; 123:252–7.
13. Hidaka F, Matsuo S, Muta T, Takeshige K, Mizukami T, Nunoi H. A missense mutation of the Toll-like receptor 3 gene in a patient with influenza-associated encephalopathy. Clin Immunol **2006**; 119:188–94.
14. Le Goffic R, Balloy V, Lagranderie M, et al. Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. PLoS Pathog **2006**; 2:e53.
15. Mibayashi M, Martinez-Sobrido L, Loo YM, Cardenas WB, Gale M Jr, Garcia-Sastre A. Inhibition of retinoic acid-inducible gene I-mediated induction of beta interferon by the NS1 protein of influenza A virus. J Virol **2007**; 81:514–24.
16. Garcia-Sastre A, Biron CA. Type 1 interferons and the virus-host relationship: a lesson in détente. Science **2006**; 312:879–82.
17. Jaatinen T, Ruuskanen O, Truedsson L, Lokki ML. Homozygous deletion of the CYP21A-TNXA-RP2-C4B gene region conferring C4B deficiency associated with recurrent respiratory infections. Hum Immunol **1999**; 60:707–14.
18. Zhang H, Zhou G, Zhi L, et al. Association between mannose-binding lectin gene polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus infection. J Infect Dis **2005**; 192:1355–61.
19. He J, Feng D, de Vlas SJ, et al. Association of SARS susceptibility with single nucleic acid polymorphisms of OAS1 and MxA genes: a case-control study. BMC Infect Dis **2006**; 6:106.
20. Davis MM, Taubert K, Benin AL, et al. Influenza vaccination as secondary prevention for cardiovascular disease: a science advisory from the American Heart Association/American College of Cardiology. J Am Coll Cardiol **2006**; 48:1498–502.
21. Roy S, Knox K, Segal S, et al. *MBL* genotype and risk of invasive pneumococcal disease: a case-control study. Lancet **2002**; 359:1569–73.
22. Burgner D, Jamieson SE, Blackwell JM. Genetic susceptibility to infectious diseases: big is beautiful, but will bigger be even better? Lancet Infect Dis **2006**; 6:653–63.